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Review

Protein Quality Control Pathways at the Crossroad of Synucleinopathies

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Abstract. The pathophysiology of Parkinson's disease, dementia with Lewy bodies, multiple system atrophy, and many others converge at alpha-synuclein (α -Syn) aggregation. Although it is still not entirely clear what precise biophysical processes act as triggers, cumulative evidence points towards a crucial role for protein quality control (PQC) systems in modulating α -Syn aggregation and toxicity. These encompass distinct cellular strategies that tightly balance protein production, stability, and degradation, ultimately regulating α -Syn levels. Here, we review the main aspects of α -Syn biology, focusing on the cellular PQC components that are at the heart of recognizing and disposing toxic, aggregate-prone α -Syn assemblies: molecular chaperones and the ubiquitin-proteasome system and autophagy-lysosome pathway, respectively. A deeper understanding of these basic protein homeostasis mechanisms might contribute to the development of new therapeutic strategies envisioning the prevention and/or enhanced degradation of α -Syn aggregates.

Keywords: Alpha-synuclein, synucleinopathies, protein homeostasis, protein aggregation, molecular chaperones, ubiquitin-proteasome system, autophagy

INTRODUCTION

Alpha-synuclein (α -Syn) was first identified in human brain extracts more than 25 years ago [1, 2], and since then many physiological roles have been ascribed to this small protein. Although α -Syn has no defined tridimensional structure in aqueous solution [3] and is soluble under most physiological conditions [4], it can adopt beta-strand rich conformations

favoring the formation of amyloid fibrils in several neurodegenerative diseases, collectively known as synucleinopathies [5–7]. For instance, α -Syn aggregates are found in distinctive neuronal structures known as Lewy bodies (LBs) and Lewy neurites (LNs) in idiopathic and familial forms of Parkinson's disease (PD) and dementia with Lewy bodies [8], and in glial cytoplasmic inclusions in multiple system atrophy [9–12]. However, instead of being able to adopt only one type of structure, recent studies revealed that aggregated α -Syn possess distinct conformations (polymorphs) with unique cytotoxicity profiles [13–17]. This suggests that different synu-

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cleinopathies arise from distinct α -Syn polymorphs, as indeed proposed by experiments in animal models [18, 19].

Although the initial events leading to α -Syn aggregation and toxicity *in vivo* are still poorly understood, several lines of evidence suggest that cellular protein quality control (PQC) pathways play a central role in these processes. Among these are the molecular chaperones and the two main protein degradation pathways, namely the ubiquitin-proteasome system (UPS) and autophagy-lysosome pathway (ALP) [20]. Here, we review basic molecular and cellular principles of α -Syn aggregation and their connection with PQC components, with a special emphasis on the suppression of α -Syn aggregation and/or toxicity by molecular chaperones.

ALPHA-SYNUCLEIN STRUCTURE AND FUNCTION

The N-terminal domain of α -Syn contains several motifs with amphipathic properties allowing for interactions with membranes (binding to lipid vesicles) and that can serve in protein-protein interactions [21] (Fig. 1). The central portion (residues 61 to 95) contains the non-amyloid-beta component of Alzheimer's disease amyloid (NAC) motif [1], which is both sufficient and required for amyloid formation [6, 22–24]. The C-terminal region has an important role in shielding the NAC motif from aggregation [6, 24]. Deletion of only the last 10 amino acids is already sufficient to accelerate α -Syn aggregation *in vitro*, and this effect is stronger upon larger C-terminal truncations up to amino acids 102–120 [24–26]. α -Syn is subject to several post-translational modifications (PTMs), including N-terminal acetylation, ubiquitylation, SUMOylation, nitration, and phosphorylation [27–32], with diverse consequences for its function and propensity to aggregate (detailed below). Several roles

have been ascribed to α -Syn, including facilitating the assembly of N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-complexes at presynaptic neuron terminals that mediate release of neurotransmitters [33, 34], and induction of membrane curvatures [35], among many others [36].

SNCA MUTATIONS REVEAL UNIQUE FEATURES OF α -SYN TOXICITY AND AGGREGATION

Two types of mutations in the *SNCA* gene have been linked to autosomal dominant forms of PD, highlighting distinct mechanisms by which α -Syn aggregation can be triggered: (i) increased gene dosage and (ii) point mutations enhancing α -Syn aggregation propensity. The latter, including A30P [37], E46K [38], H50Q [39, 40], G51D [41], and A53T [42], A53V [43], and A53E [44] (see Fig. 1), have been discovered by genetic screens in families with hereditary PD and directly influence α -Syn aggregation to different extents and via discrete pathways [45]. Mutants such as α -Syn^{A53T} largely enhance α -Syn aggregation into fibrils [45, 46], most likely by changing the conformational landscape that α -Syn populates towards aggregation-prone conformers, without disrupting vesicular interactions [21]. In contrast, the A30P mutation does not markedly modulate α -Syn aggregation compared to overexpression of α -Syn^{WT} in cellular models [46–48]. Instead, it abolishes α -Syn interaction with lipid vesicles both *in vitro* [21, 49] and *in vivo* [50], which may lead to a buildup of cytosolic α -Syn levels, and eventually contributes to α -Syn aggregation. This implies that α -Syn aggregation is also extremely dependent on its concentration and can even be triggered by the wild type protein [50, 51]. In fact, familial PD cases caused by duplications or triplications of the *SNCA* locus have been identified [52–56],

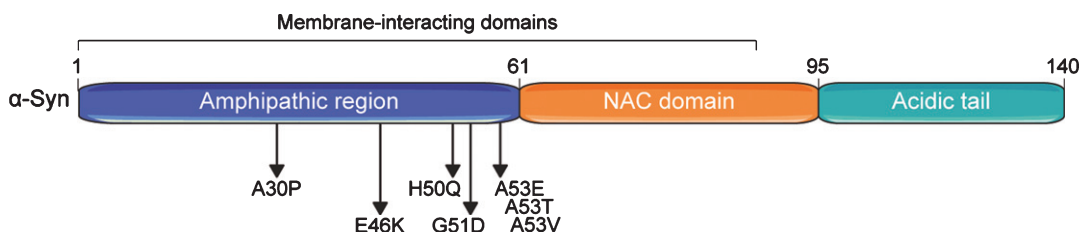


Fig. 1. Domain structure of the human alpha-synuclein (α -Syn) protein. α -Syn comprises three basic domains: an N-terminal amphipathic region, a central non- β -amyloid component (NAC) domain, and a C-terminal acidic domain. Seven membrane-interacting amino acid motifs are also present in the first half of the protein. The region preceding the NAC domain concentrates all pathogenic α -Syn mutations identified so far. Numbers on the upper part of the structure refer to amino acid positions.

with increased gene dosage correlating with earlier age at onset of disease [57].

MODELLING α -SYN AGGREGATION: SEEDED VERSUS NON-SEEDED CONDITIONS

As for any aggregation-prone protein, α -Syn molecules adopt conformations that allow the establishment of non-native interactions between molecules and their coalescence into thermodynamically unstable assemblies [58]. It is upon conformational transition to more regular and complementary interfaces [59] that stable seeds are generated, capable of acting as conformational template of the amyloid state [58]. In contrast, the highly stable preformed α -Syn aggregates commonly used in studies grow by incorporation of α -Syn molecules to their ends, as the binding of additional molecules to fibrillar ends generates an incorporation site for another molecule [58]. The spontaneous aggregation of α -Syn into amyloid fibrils thus is a multi-step process during which various intermediates are generated that provide copious opportunities for PQC interference.

The exogenous provision of preformed fibrils (seeded aggregation) bypasses the initial requirement for seed formation and allows the rapid incorporation of α -Syn monomers to their ends [58], presenting a more limited number of conformational states at which PQC components can interfere. The molecular mechanisms of chaperone modulation of α -Syn aggregation in spontaneous *versus* seeded aggregation are thus likely to differ significantly. Indeed, some chaperones interfere with unseeded aggregation (e.g., DNAJB6 [60]), whilst others selectively act on the elongation of preformed seeds (e.g., HSPB5 [61]).

The distinction between unseeded and seeded α -Syn aggregation is thus extremely important to our understanding of the α -Syn aggregation process and PQC effects thereon. Cellular studies aimed at investigating PQC components in α -Syn aggregation are most often unable to clearly determine whether unseeded, seeded or both processes are targeted and to what extent.

Non-seeded α -Syn aggregation

It has been surprisingly difficult to consistently model spontaneous, non-seeded α -Syn aggregation in cellular and organismal models. In fact, recent nuclear magnetic resonance data showed that α -Syn

at physiological concentrations remains largely in a monomeric, highly dynamic state in cells [4]. Since the crowded cellular environment is expected to facilitate α -Syn aggregation, these data strongly suggest the existence of agents (such as molecular chaperones and protein degradation machineries) that efficiently counteract α -Syn aggregation under normal circumstances.

To date, most studies investigating *de novo*, non-seeded, α -Syn aggregation have relied on overexpression of either wild-type (WT) or mutant variants of α -Syn. In one of the earliest models, α -Syn inclusion formation was detected in human neuroglioma H4 cells and mouse primary cortical neurons only upon overexpression of α -Syn constructs (α -Syn^{WT}, α -Syn^{A30P}, or α -Syn^{A53T}) harboring distinct C-terminal tags of variable sizes, which affected proteasomal clearance [47]. Since untagged α -Syn variants remained soluble, the tag potentiated aggregation probably through the exposure of the NAC region. Others have employed the co-expression of α -Syn with distinct aggregation-prone proteins that co-localize with α -Syn in LBs to trigger inclusion formation, such as synphilin-1 [45, 62–65] and tubulin polymerization-promoting protein (TPPP/p25 α) [66], but it is not entirely clear whether these are indeed active drivers of α -Syn aggregation. Some studies have also used bimolecular fluorescence complementation assays to assess *de novo* α -Syn aggregation [45, 67, 68]. In these cases, fluorescence is reconstituted and detected upon co-expression of two α -Syn constructs fused to either the N- or C-terminus halves of a fluorescent protein (for example, the split Venus-system). However, it is still neither clear whether such assemblies are of fibrillar nature, as the interaction of little as two α -Syn molecules is already sufficient to reconstitute fluorescence, nor to what extent the reconstitution of the functional fluorescent protein drives assembly. Nevertheless, some degree of α -Syn fibrillation was detected upon overexpression of distinct split Venus- α -Syn in flies [68]. In any case, true detergent-insoluble α -Syn aggregates are either usually not observed in unseeded α -Syn models, or they comprise only a small fraction of the total α -Syn pool, highlighting the urgent need for better models to document α -Syn aggregation.

Seeded α -Syn aggregation

Seeded aggregation experiments have been instrumental in uncovering many of the basic principles

of α -Syn pathology (see for instance [26, 69–71]). Indeed, most of the α -Syn literature relies on experiments in which an exogenous source of α -Syn amyloid fibrils is administered to cells or animals in order to trigger aggregation of the endogenous α -Syn (i.e., the α -Syn pool generated by cells *de novo*, even if it consists of an artificial transgene). In these cases, exogenous α -Syn fibrils come from either *in vitro* reactions using recombinant α -Syn [69] or from fibrils isolated from animal models or human post-mortem tissue [19, 26, 72]. As stated above, structural differences of α -Syn fibrils may lead to different synucleinopathies [15, 73]. However, it should be noted that there is currently no evidence demonstrating that human pathology starts upon exposure to exogenous α -Syn seeds [36], suggesting that factors such as cellular stress may trigger the formation of the first α -Syn seeds.

α -SYN AGGREGATION AND TOXICITY IN THE CONTEXT OF PQC SYSTEMS

Molecular chaperones

Suppression of α -Syn aggregation by Hsp70 machines

Molecular chaperones are at the heart of several PQC pathways and have been extensively implicated as protective agents against protein aggregation and neurodegeneration [74]. Here, we will primarily focus on the action of Hsp70 machines on α -Syn aggregation and toxicity. The human genome encodes for multiple Hsp70 isoforms and these Hsp70s act with the help of many co-factors a system that we refer to as the Hsp70 machines.

Purified Hsp70s (e.g., HSPA1A or HSPA8) alone can almost completely block α -Syn fibrillation at substoichiometric ratios, generating small aggregates composed of both proteins [25, 75–77]. Interestingly, addition of recombinant Hsp70-interacting protein (Hip) to reactions containing Hsp70 and monomeric α -Syn completely blocked Hsp70 co-aggregation and led to sustained inhibition of α -Syn aggregation in an ATP-dependent manner [78], highlighting the importance of additional co-factors for maximal suppression of α -Syn aggregation by Hsp70 machines (see below). Purified Hsp70s (HSPA1A or HSPA8) have been shown to bind a range of α -Syn assemblies, including monomers [77], pre-fibrillar [76, 78], and fibrillar species [75, 79, 80]. α -Syn amino acid stretches that are bound by Hsp70s span residues 10–45 and 97–102 [77].

Besides the suppression of α -Syn nucleation, Hsp70s also bind to α -Syn seeds [75] and prevent fibril elongation [79, 80]. These latter findings are consistent with a holdase function of Hsp70s against α -Syn fibril elongation, possibly shielding fibrillar ends from further incorporation of α -Syn molecules [79, 80].

In cells, co-expression of Hsp70 decreased the amount of high molecular weight α -Syn species [64, 65], probably by stabilization of α -Syn in assembly-incompetent states [81]. This could account for decreased cytotoxicity of α -Syn upon overexpression of Hsp70 [65, 82]. Indeed, Hsp70 overexpression in primary neurons markedly decreased the size, but not the amount, of secreted α -Syn species [82]. Since Hsp70 was also detected in the culture medium, it was proposed to bind monomeric or low molecular weight pre-fibrillar α -Syn assemblies and prevent further aggregation into mature fibrils [82].

At the organismal level, mice overexpressing both α -Syn and the rat HspA1 showed a 2-fold reduction in 1% Triton-X100-insoluble α -Syn-containing aggregates, compared to animals expressing α -Syn only [65]. In the fruit fly *Drosophila melanogaster*, selective expression of α -Syn^{WT}, α -Syn^{A30P}, or α -Syn^{A53T} in dopaminergic neurons for 20 days led to a 50% cell loss, but this could be fully rescued by targeted co-expression of the human Hsp70 isoform HSPA1L [83]. Interestingly, despite its cytoprotective effects, HSPA1L did not inhibit α -Syn inclusion formation, but rather co-localized with α -Syn in LB-like structures, suggesting that Hsp70 binding reduced toxic interactions of α -Syn with other biomolecules. Such phenomenon is conserved from flies to humans, with evidence for the accumulation of not only Hsp70, but also its cochaperones Hsp40/DNAJs and Hsp110/NEFs, into LBs and LNs from patients with PD, dementia with Lewy bodies, and other synucleinopathies [63, 83]. Indeed, the titration of Hsp70s out of solution by misfolded α -Syn has been hypothesized to contribute to disease onset due to lowering of the functional pool of Hsp70 available for protein quality control pathways [78, 84].

In vitro, the suppression of α -Syn aggregation by either Hsp70 (HSPA1A) or Hsc70 (HSPA8) does not require ATP/ADP cycling [25, 75, 78, 80], nor cochaperones, such as DNAJB1 [78]. In fact, DNAJB1, which stimulates Hsp70 cycling, even counteracts such sequestering activity [76]. However, these factors are essential for the proper function of Hsp70 in quality control pathways *in vivo* [85]. Indeed, overexpression of other members from the family of

Hsp70 co-chaperones also successfully prevents α -Syn aggregation and/or toxicity in cell and mouse models of PD. Of special relevance in this context is the large family of Hsp40/DNAJ proteins, which are regarded as the main determinants of specificity of Hsp70 machines, since different DNAJs bind to distinct client proteins and deliver those to Hsp70 [85, 86]. Thus, DNAJs could be exploited to maximize the activity of Hsp70 machines towards specific substrates. For example, besides inhibiting α -Syn aggregation *in vitro* [76], DNAJB1 almost completely abolished α -Syn inclusion formation in cells overexpressing α -Syn [63]. This has also been shown for DNAJB6 and its close homologue DNAJB8 [51], both of which also strongly suppress the aggregation of other amyloidogenic polypeptides, including expanded polyglutamine-containing proteins [60, 87] and the amyloid-beta protein [88]. Interestingly, α -Syn aggregation was not suppressed by a DNAJB6 mutant that does not interact with Hsp70 [51], strengthening the notion that cooperation between distinct components of Hsp70 machines is essential for optimal function. Despite these examples, little is still known on the contribution of different DNAJs and/or NEFs to the Hsp70-dependent suppression of α -Syn aggregation *in vivo*. Similar to recently developed *in vitro* screens for inhibiting tau aggregation [89], or enhancing α -Syn disaggregation (see below) [90], further comparative studies using distinct compositions of Hsp70 machines are urgently required to better understand and manipulate Hsp70 machines in synucleinopathies.

Disaggregation of α -Syn fibrils by Hsp70 machines

The diversity and complexity of Hsp70 machines is also highlighted by studies investigating the potential of these systems to disaggregate pre-existing α -Syn amyloids. For instance, although Hsp70 alone does not modify or disaggregate mature α -Syn fibrils at relevant time-scales *in vitro* [75, 91], a specific Hsp70/HSPA-Hsp40/DNAJ-Hsp110/NEF combination showed powerful, ATP-dependent disaggregase activity against α -Syn amyloids [90]. Indeed, optimal fragmentation and depolymerization of α -Syn fibrils was detected upon combining Hsc70/HSPA8 with Hdj1/DNAJB1 and the NEF Apg2/HSPA4, but not upon addition of other Hsp70 machine members, such as Hsp70/HSPA1A, DNAJA1, DNAJA2, or BAG1. Moreover, a precise stoichiometry between these components was crucial for productive disaggregation [90, 91], further illustrating the tight balance

between specificity and levels of chaperones/co-chaperones for the activity of Hsp70 machines. It is still not known whether Hsp70-mediated disaggregation of α -Syn also occurs *in vivo*, but it is tempting to speculate that the breakup of fibrils into smaller, more soluble assemblies facilitates their processing by downstream PQC components, as discussed below. However, it is equally possible that disaggregation could be detrimental and facilitate α -Syn seed propagation. Further studies are necessary to clarify these issues.

Clearance of α -Syn assemblies via protein degradation machineries

The two major cellular protein degradation machineries comprise the UPS and ALP, with the latter encompassing both autophagosome-dependent and independent pathways [92]. There is an intricate and tightly regulated crosstalk between proteasomal and lysosomal pathways engaged in the processing of α -Syn, as several studies reported preferential degradation of α -Syn via the UPS or ALP [31, 93–98]. Moreover, α -Syn (WT or distinct mutants) overexpression can impair the activity of both the UPS [99, 100] and distinct components of the ALP [66, 101–104], which would act in a progressive pathogenic feedback loop to accelerate aggregation and toxicity. Whether UPS or ALP lead to the degradation of α -Syn assemblies is still actively debated. Recent findings suggest, however, that the UPS has a more prominent role in degrading smaller α -Syn assemblies at least when protein quality systems are highly active, as is generally the case in young, healthy organisms [99]. Autophagic activity seems to be more required for larger α -Syn assemblies and upon increased α -Syn burden, due to either mutations that lead to α -Syn accumulation or decreased activity of other PQC components, as observed with aging [99]. α -Syn has also been shown to be recognized and degraded by other cellular (extracellular) proteases not directly linked to the UPS and ALP pathways [105, 106]. However, the extent to which such enzymes are required for proper α -Syn turnover and/or inhibition of propagation is still poorly understood.

PTMs also play a role in α -Syn processing and act as important sorting hubs to distinct protein degradation machineries. For instance, the covalent binding of ubiquitin to α -Syn, via either mono-(monoUb) or polyubiquitylation (polyUb) in distinct linkage types, has opposing consequences to the

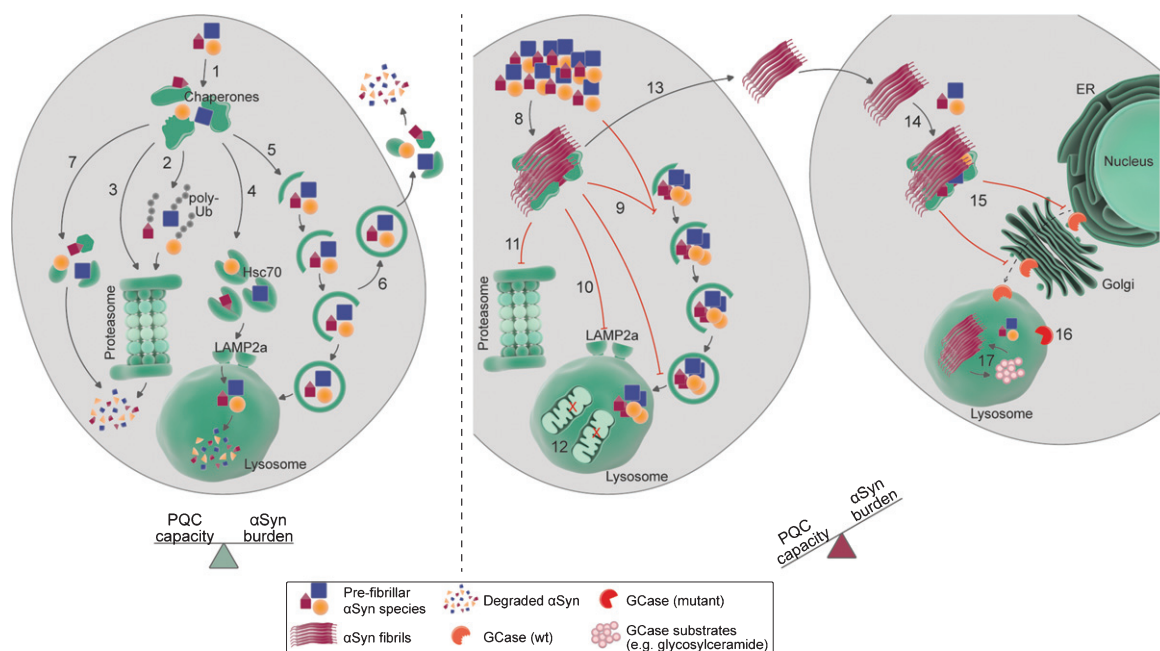


Fig. 2. Targeting and processing of alpha-synuclein (α -Syn) by protein quality control (PQC) pathways. *Left*: in normal conditions, in which the cellular PQC capacity is in balance with the α -Syn burden, soluble as well as pre-fibrillar α -Syn assemblies (after disassembly) have been shown to be targeted to and degraded by several PQC components. The initial survey of α -Syn species might be performed by molecular chaperones (1), which can facilitate the sorting of α -Syn to distinct degradative routes, such as the ubiquitin-proteasome system (UPS; 2), a ubiquitin-independent proteasomal degradation pathway (3), chaperone-mediated autophagy (CMA; 4), macroautophagy (5), secretion via endosomes (6) [162], and proteolytic digestion by intracellular (7) or extracellular proteases. Fibrillar α -Syn assemblies can trap several biomolecules, including molecular chaperones (8), which contributes to chaperone depletion and decreases PQC capacity. Similarly, α -Syn aggregation has been linked to impairment of different steps of macroautophagy (9), CMA (10), and proteasomal degradation (11). In some experimental setups, increased α -Syn levels can also lead to increased autophagic flux and destruction of organelles, such as mitochondria (12). α -Syn species can also be secreted to the extracellular space and taken up by neighboring cells (13), where they seed the aggregation of soluble α -Syn species (14). α -Syn aggregation additionally impairs the intracellular trafficking of other proteins, such as the lysosomal enzyme glucocerebrosidase (GCase; 15). Decreased lysosomal GCase activity, due to either mislocalization of wildtype (wt) GCase or mutant GCase variants (16), leads to accumulation of GCase substrates (such as glycosylceramide; 17), which might potentiate α -Syn aggregation. See main text for further mechanistic details and references. ER: endoplasmic reticulum; Hsc70: heat shock cognate 71 kDa protein; LAMP2a: lysosome-associated membrane protein 2 isoform a; poly-Ub: poly-ubiquitin.

fate of α -Syn. For instance, the co-chaperone CHIP (carboxyl terminus of Hsp70-interacting protein), a ubiquitous E3 Ub-ligase and crucial downstream effector of Hsp70 machineries [85], was shown to promote α -Syn degradation via both the UPS and ALP [107]. Also, while monoubiquitylation by the E3 ubiquitin-ligase SIAH targeted α -Syn to the UPS, removal of the ubiquitin moiety by the deubiquitylase USP9X favored α -Syn degradation via macroautophagy [108]. Yet another ubiquitin-ligase (Nedd4) facilitated the binding of K63-linked polyUb chains to α -Syn and promoted its lysosomal degradation via the ESCRT pathway [109]. Depending on its assembly state, other PTMs such as SUMOylation, phosphorylation, nitration, O-GlcNAcylation, oxidation, and dopamine-modification can also modulate

α -Syn processing via downstream degradation pathways [30, 31, 110–114]. In this context, the main findings associated to the partition of α -Syn between the UPS and ALP are discussed below and illustrated in Fig. 2.

Ubiquitin-proteasome system

In mammalian cells, the central player of the UPS is the 26S proteasome, a large, ATP-dependent multi-protein complex devoted to the selective destruction of target proteins [115]. Evidence for the degradation of α -Syn via proteasomes comes from both *in vitro* [116] and cellular studies [117–119], with not only monomeric, but maybe also pre-fibrillar α -Syn species (after dissociation) being targeted to this pathway [30, 100]. Several Ub-ligases interact with

α -Syn and catalyze the addition of either mono- or polyUb chains with either cytoprotective or toxic consequences depending on the specific experimental setup, presumably due to differential impact on cellular α -Syn half-life [109, 117, 120, 121]. Unmodified α -Syn can also be degraded by proteasomes via an Ub-independent pathway [118], particularly relevant for phosphorylated α -Syn at serine 129 (α -Syn^{PS129}) [119]. A mutant mimicking α -Syn^{PS129} (α -Syn^{S129E}) was shown to be a poor autophagic substrate [111], re-emphasizing the complementary importance of the different degradation pathways. Several lines of evidence also suggest that an increased α -Syn burden inhibits proteasomal activity, which in turn might lead to a further increase in α -Syn levels, thus establishing a pathogenic feedback loop favoring α -Syn aggregation [100, 104, 122, 123].

Autophagy-lysosome pathway

The numerous reports on α -Syn degradation via the ALP highlight the importance of lysosomal-dependent regulation of α -Syn levels [124]. Not surprisingly, a plethora of therapeutic strategies targeting the ALP have been explored to tackle α -Syn aggregation and toxicity (reviewed in [125]). The ALP comprises catabolic processes that converge at the lysosome, being usually divided in three distinct types: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) [126]. Macroautophagy relies on the engulfment of substrates within autophagosomes, which are double-layered membrane vesicles that sequester intracellular components and target them to lysosomes for degradation [127]. Most long-lived proteins, protein aggregates and even whole damaged organelles are degraded via macroautophagy [92, 128]. The importance of macroautophagy for normal cellular function is exemplified by experiments in which loss of macroautophagy in neurons led to accumulation of ubiquitylated proteins and inclusion bodies, and neurodegeneration [129]. Moreover, mutations in different autophagy-related genes, such as *ATG5*, lead to genetic diseases with neurologic phenotypes in humans [130].

Data supporting a role for macroautophagy in the degradation of monomeric and pre-fibrillar α -Syn assemblies come mainly from studies detecting α -Syn buildup upon exposure of cell lines overexpressing either WT or mutant α -Syn variants to the inhibitor of autophagosome formation 3-methyladenine [93, 95, 97, 131]. *In vivo*, overexpression of beclin-1, which is involved in autophagosome

formation via the phosphatidylinositol 3-phosphate kinase complex, rescued neurological deficits of α -Syn transgenic mice [131]. Yet, beclin-1 is involved in other endosomal pathways, not directly linked to macroautophagy [132], which may contribute to the reduction of α -Syn levels and improved performance of animals overexpressing α -Syn [131]. Impairment of lysosomes, toward which all ALP components converge, with bafilomycin A1 also resulted in α -Syn buildup, further supporting a role for the ALP in α -Syn degradation [96, 125, 133, 134]. Nonetheless, whether macroautophagy is capable of degrading aggregated, insoluble α -Syn assemblies, such as those present in LBs, is still debated. For instance, in a cell model of endogenous α -Syn aggregation upon exposure to exogenous α -Syn pre-formed fibrils, α -Syn inclusion resisted lysosomal degradation [134]. In addition, increasing macroautophagy flux upon α -Syn overexpression was also shown to have detrimental effects, ranging from increased degradation of mitochondria (mitophagy) in both cellular [135], and animal models of PD [136] to enhanced secretion of α -Syn assemblies to the extracellular space [66], that may contribute to the spreading of pathogenic α -Syn aggregates. On the other hand, Gao and colleagues (2019) have recently demonstrated enhanced degradation of internalized exogenous α -Syn pre-formed fibrils in neuronal cell lines upon treatment with different autophagy inducers, suggesting that lysosomes might be capable of clearing seeded fibrillar α -Syn [137].

Different from macroautophagy, CMA encompasses the selective targeting of substrates to lysosomes via Hsc70 (HSPA8) and its co-chaperones, to specifically recognize cargo proteins with a KFERQ-like pentapeptide motif, and lysosomal-associated membrane protein 2a (LAMP2a)-mediated substrate translocation across lysosomal membranes [138, 139]. Several lines of evidence support the involvement of CMA in the processing of α -Syn [125]. In an *in vitro* lysosomal reconstitution assay, α -Syn^{WT} was selectively targeted to lysosomes by LAMP2a, and mutations within a KFERQ-like motif in α -Syn C-terminus abolished this activity [94]. In cultured cells overexpressing α -Syn, macroautophagy inhibition led to higher α -Syn clearance via CMA [95, 140], while α -Syn protein levels were increased upon specific knockdown of LAMP2a [141], or HSPA8 [141, 142]. Compared to healthy controls, lower LAMP2a protein levels were detected in brains from early-stage PD patients, accompanied by a buildup of α -Syn and other known CMA sub-

strates, such as myocyte-specific enhancer factor 2D (MEF2D) and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (I κ B α) [143]. The importance of CMA in processing α -Syn monomers and dimers, but not pre-fibrillar assemblies [111], is somewhat diminished by the finding that α -Syn steady-state levels were unchanged in *Lamp2* knockout mice [144]. This however may be due to developmental adaptations in other PQC components, such as the UPS, as outlined above, thus masking the influence of CMA. Indeed, *in vivo* down-regulation of *Lamp2a* in rats resulted in accumulation of ubiquitin-positive α -Syn inclusions in the *substantia nigra* followed by loss of dopaminergic neurons [145]. Additional evidence for CMA involvement in α -Syn degradation comes from observations that distinct PTMs, including oxidation, nitration, and modification by oxidized dopamine, impair α -Syn degradation via CMA, resulting in its buildup [111]. Importantly, similar to the rare α -Syn^{A30P} and α -Syn^{A53T} mutations [94], dopamine-modified α -Syn (present in sporadic PD cases) also interferes with the processing of other CMA substrates [111], further contributing to the imbalance of protein homeostasis.

Upon convergence of distinct ALP routes at lysosomes, soluble α -Syn assemblies can be degraded by acidic proteases, such as cathepsin D [146–148]. Another lysosomal enzyme that has attracted much attention in synucleinopathies is glucocerebrosidase (GCase). While homozygous mutations in the GCase-encoding gene *GBA1* cause Gaucher's disease [149], heterozygous mutations are a well-established risk factor for developing PD [150]. Indeed, α -Syn buildup is observed in several models of GCase deficiency. This occurs upon pharmacological inhibition of GCase activity in cultured cells [151, 152] and also in *GBA1* mutant backgrounds, both in mouse models overexpressing α -Syn [153–155] and in PD patient iPS-derived dopaminergic neurons [156, 157]. α -Syn buildup impairs GCase trafficking and targeting to lysosomes [158, 159]. Conversely, rescue of GCase activity in mice overexpressing α -Syn^{A53T} reduced α -Syn levels and toxicity [155], establishing a pathogenic feedback loop that promotes loss of GCase function, and α -Syn accumulation, aggregation and, potentially, cell-to-cell transmission of α -Syn seeds [160, 161]. Altogether, these results suggest that the upregulation of autophagy without a simultaneous improvement of lysosomal capacity might not be a true therapeutic strategy in synucleinopathies.

CONCLUDING REMARKS

The topics discussed here paint a complex picture of cellular strategies engaged in the tight regulation of α -Syn protein levels, which ultimately determine its aggregation propensity and associated toxicity. Even though there are still some fundamental gaps in our understanding of α -Syn biology, it has become increasingly clear that the activity of dedicated PQC components, such as molecular chaperones, the UPS, and ALP is a crucial line of defense against α -Syn-mediated pathology. Failure of these systems (e.g., due to cellular stress, genetic predisposition, or aging) will influence α -Syn levels and solubility, eventually leading to disease. However, it is tempting to envision that novel therapeutic strategies to prevent, slow down and/or halt progression of synucleinopathies will emerge based on our understanding of protein homeostasis in general and in particular in components that prevent initiation of α -Syn protein aggregation or help clearing them before they affect neuronal health and synaptic integrity.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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